Allelic Loss in Esophageal Squamous Cell Carcinoma Patients with and without Family History of Upper Gastrointestinal Tract Cancer

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ABSTRACT

Chromosomal regions with frequent allelic loss may point to major susceptibility genes that will assist in understanding molecular events involved in esophageal carcinogenesis. Esophageal squamous cell carcinoma samples and blood from 46 patients, including 23 patients with and 23 patients without a family history of upper gastrointestinal cancer, were screened using laser microdissected DNA and tested for loss of heterozygosity (LOH) at 18 marker loci representing 14 chromosomal regions (on 2q, 3p, 4p, 4p, 5q, 6q, 8p, 9p, 9q, 11p, 13q, 14q, 15q, and 17p) identified in an earlier genome-wide scan to have frequent LOH. Clinical/pathological and lifestyle risk factor data were also collected. For all 46 tumors combined, the lowest frequency LOH for any of the 18 markers was 37%, and 8 markers showed LOH in ≥75% of informative tumors. One marker (*D13S894* on 13q) showed greater LOH in patients with a positive family history (93% *versus* 50%; $P = 0.04$), whereas two markers (*D6S1027* on 6q and *D9S910* on 9q) had significantly more LOH in patients with metastasis, and one marker (*D4S2361* on 4p) showed significantly higher LOH in patients with a lower pathological tumor grade. No relation was seen between LOH and lifestyle risk factors. This study confirms the previously observed high frequency LOH for these 14 chromosomal regions, including a locus on 13q where LOH is more common in patients with a family history of upper gastrointestinal cancer than in those without such history, suggesting that a gene in this area may be involved in genetic susceptibility to esophageal cancer.

INTRODUCTION

Esophageal squamous cell carcinoma is one of the most common fatal cancers worldwide. There is great geographic variation in the occurrence of this tumor, including exceptional high risk areas such as Shanxi province, a region in north central China with some of the highest esophageal cancer rates in the world (1–4). Although epidemiological studies indicate that tobacco and alcohol are the major risk factors for esophageal cancer in the low-risk regions of Europe and North America, the etiology in high-risk areas remains less clear. Several possibilities, including nitrosamines, nutritional deficiencies, fermented and moldy foods, and the exposure to polycyclic aromatic hydrocarbons have been considered, but none have been convincingly linked to Shanxi's high rates of esophageal cancer (5). Previous studies in this high-risk region have, however, demonstrated a strong tendency toward familial aggregation (6–10), suggesting that genetic susceptibility may play a role in the etiology of esophageal cancer.

The molecular events associated with the initiation and progression of esophageal squamous cell carcinoma remain poorly understood, although frequent allelic deletions and other genetic abnormalities affecting individual tumor suppressor genes have been detected in these tumors (11, 12). Chromosomal regions with frequent allelic loss may point to major susceptibility genes that will assist in understanding molecular events involved in esophageal carcinogenesis and serve as the basis for the development of markers for genetic susceptibility testing and screening for the early detection of this cancer.

To better understand the genetic changes involved in the development of esophageal cancer and ascertain potential susceptibility genes, we had previously conducted a genome-wide scan in 11 esophageal squamous cell carcinoma patients with a family history of upper gastrointestinal cancer (*i.e.*, esophageal or stomach cancer) using 366 microsatellite markers. In that scan, 14 chromosomal regions were identified by 46 markers having very high frequency (≥75%) LOH.[2,3] The study reported here expands our efforts to understand the role of genetics in the etiology and prevention of esophageal cancer in the

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[2] The abbreviation used is: LOH, loss of heterozygosity.

[3] N. Hu, M. Roth, M. Polymeropolous, Z. Z. Tang, M. R. Emmert-Buck, Q. H. Wang, A. M. Goldstein, S. S. Feng, S. M. Dawsey, T. Ding, Z. P. Zhuang, X. Y. Han, T. Ried, C. Giffen, and P. R. Taylor. Identification of novel regions of allelic loss from a genome-wide scan of esophageal squamous cell carcinoma in a unique high-risk Chinese population, submitted for publication.

high-risk region of Shanxi, China by confirming LOH in the 14 chromosomal regions identified in our initial genome-wide scan and by examining the relation of LOH to family history of upper gastrointestinal cancer, clinical/pathological characteristics, and lifestyle risk factors. A total of 46 esophageal squamous cell carcinoma cases (23 with and 23 without a family history of upper gastrointestinal cancer) were screened for LOH at 18 marker loci using laser microdissected DNA.

MATERIALS AND METHODS

Patient Selection. Patients presenting in 1995 and 1996 to the Shanxi Cancer Hospital in Taiyuan, Shanxi province, People's Republic of China, who were diagnosed with esophageal cancer and were considered candidates for curative surgical resection were identified and recruited to participate in this study. The study was approved by the Institutional Review Boards of the Shanxi Cancer Hospital and the United States National Cancer Institute. For this study, a total of 46 patients with esophageal cancer were selected who had a histological diagnosis of esophageal squamous cell carcinoma confirmed by pathologists at both the Shanxi Cancer Hospital and the National Cancer Institute. None of the patients had prior therapy, and Shanxi was the ancestral home for all. Of the 46 esophageal cancer patients studied, the 23 patients in group 1 had a family history of upper gastrointestinal cancer (*i.e.,* one or more first-, second-, or third-degree relatives with cancer of the esophagus or gastric cardia), and the 23 patients in group 2 had no family history of upper gastrointestinal or other cancer. Esophageal and gastric cardia cancers were combined for family history because historically both were diagnosed based on dysphagia, and because their coexistence at very high rates in the same geographic region strongly suggests common environmental and genetic etiologies.

After obtaining informed consent, patients were interviewed to obtain information on demographic and lifestyle cancer risk factors, including tobacco use (yes/no), frequency of alcohol consumption (never, rarely, weekly, daily), frequency of pickled vegetable consumption (never, monthly, weekly, daily), frequency of hot (temperature) food consumption (never, monthly, weekly, daily), and a detailed family history of cancer (including all cancers in first-, second-, and third-degree relatives). Data were also recorded concerning the clinical/pathological characteristics of the patients' tumor, including location (upper, middle, lower third), pathological grade (G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated), pathological Tumor-Node-Metastasis stage (I–IV), and lymph node metastasis (yes/no).

Biological Specimen Collection and Processing. A 10-ml sample of venous blood was drawn from each patient prior to surgery, and genomic DNA was extracted and purified. Tumor tissue obtained during surgery was fixed in ethanol and embedded in paraffin.

Laser Microdissection and Extraction of DNA. Tumor cells were microdissected under direct light microscopic visualization using methods described previously (13, 14). Briefly, unstained, ethanol-fixed, paraffin-embedded 5-μm histological tissue sections were prepared on glass slides, deparaffinized twice with xylene, rinsed twice with 95% ethanol, stained with eosin, and air-dried. Specific cells of interest were selected from the eosin-stained slides and microdissected by laser capture microdissection (Pixcell 100; Arcturus Engineering, Mountain View, CA). Procured cells were immediately resuspended in an 80-μl solution containing 0.01 M Tris-HCl, 1 mM EDTA, 1% Tween 20, and 0.1 mg/ml proteinase K (pH 8.0) and incubated two nights at 37°C. The mixture was then boiled for 5 min to inactive the proteinase K. Two μl of this solution were used for each PCR reaction.

Markers, PCR, and LOH Reading and Interpretation. Eighteen polymorphic fluorescein-labeled microsatellite markers from chromosomes 2q, 3p, 4p, 4q, 5q, 6q, 8p, 9p, 9q, 11p, 13q, 14q, 15q, and 17p, with heterozygosity ranging from 61 to 84%, were used for this study (Cooperative Human Linkage Center Human Screening Version 8, Research Genetics, Inc., Huntsville, AL). The majority of these markers contain tetranucleotides ($n = 14$), and the remainder contain trinucleotides ($n = 4$; Table 2).

These 18 markers were selected to represent the 14 chromosomal regions identified from the 46 markers with very high frequency LOH in our earlier genome-wide scan.

DNA extracted from tumor cells microdissected from the resection specimen and genomic DNA from venous blood were used for each patient. PCR reactions were carried out using a 10-μl final volume containing 1.0 μl of 10× PCR buffer II [100 mM Tris-HCl (pH 8.3), 500 mM KCL], 1.0 μl of 1.25 mM deoxynucleotide triphosphate, 0.6 μl of $MgCl_2$, 2 μl of DNA extraction buffer, 0.6 μl of each primer, and 0.09 μl of AmpliTaq DNA polymerase (Perkin-Elmer). The amplifications were performed using a Techne/Genius thermal cycler for 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. An elongation step at 72°C for 10 min was added to the final cycle. The PCR products were mixed with 5 μl of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol) and were denatured for 6 min at 95°C and chilled on ice until loaded onto a 6% polyacrylamide gel. Samples were electrophoresed at 25 W for 1–3 h.

The gels were scanned by SCANNER (Molecular Dynamics, Model 575; scanner type, FluorImager SI; Image analysis software, Image QuanNT, version 3.51, 1995). LOH was defined as complete or near complete loss of a band in the tumor sample relative to the corresponding normal DNA. Microsatellite instability was defined as the presence of an extra band (allele) in the tumor DNA not seen in the normal DNA. Convincing evidence of a homozygous deletion in a tumor sample was not observed at any of the 18 markers used. All informative cases were repeated to confirm the findings. Two reviewers independently read all results from computer printouts, and all positives identified by either of the two initial readers were confirmed by a third reader.

Calculation of the Frequency of Allelic Loss. The frequency of allelic loss at each chromosome locus was calculated as the number of tumors with allelic loss at that locus divided by the number of informative tumors at that locus. The frequency of allelic loss at each chromosome locus was classified as low (0–24%), medium (25–49%), high (50–74%), or very high (≥75%).

Statistical Analysis. The frequency of allelic loss was compared in group 1 and group 2 by the χ^2 or Fisher's exact test.

The association between the frequency of allelic loss and risk factors or clinical characteristics was evaluated by the *t* test (for continuous variables) or the χ^2 or Fisher's exact test (for nominal variables), and significant dichotomous outcomes were expressed as an odds ratio with a 95% confidence interval.

RESULTS

Patient Characteristics. A total of 46 esophageal squamous cell carcinoma patients, including 27 males and 19 females, were evaluated (Table 1). Group 1 (positive family history for upper gastrointestinal cancer) included 18 patients with cancer in a first-degree relative, 4 with cancer in a second-degree relative, and 1 with cancer in a third-degree relative. Ten patients had more than one affected relative.

There were no significant differences between groups 1 and 2 for age (54.2 *versus* 53.7 years; $P = 0.43$), gender (53% *versus* 65% male; $P = 0.55$), tumor location (lower = 26% *versus* 13%, middle = 65% *versus* 83%, other = 9% *versus* 4%; $P = 0.41$), tumor grade (G1 = 17% *versus* 9%, G2 = 74% *versus* 78%, G3 = 9% *versus* 13%; $P = 0.64$), tumor stage (stage 2 = 0% *versus* 9%, stage 3 = 100% *versus* 91%; $P = 0.49$), lymph node metastasis (32% *versus* 52%; $P = 0.23$), tobacco use (43% *versus* 65%; $P = 0.24$), any alcohol consumption (52% *versus* 65%; $P = 0.55$), any pickled vegetable consumption (96% *versus* 96%; $P = 1.00$), or any hot food consumption (91% *versus* 78%; $P = 0.41$; Table 1).

Microsatellite Instability. Microsatellite instability was observed in only four patients in group 1 (SHE150 at locus *D4S2632*; SHE328 and SHE495 at locus *D3S4545*; and SHE409 at locus *D2S434*) and in one patient in Group 2 (SHE200 at locus *D5S2501*).

Family History of Upper Gastrointestinal Cancer and Frequency of LOH. Table 2 and Fig. 1 show the locations and characteristics of the 18 microsatellite markers and the frequency of allelic loss of these markers in the 46 patients studied. Allelic loss in informative tumors was common for the 18 markers tested overall (69%) and tended to be more common in those with a family history of upper gastrointestinal cancer compared to those without a family history of upper gastrointestinal cancer (72% *versus* 66%; $P = 0.12$). A higher frequency of LOH was observed in group 1 than in group 2 for loci on chromosomes 4p (*D4S2632*), 4q (*D4S2361*), 5q (*D5S2501*), 8p (*D8S1106*), 9p (*GATA62F03*), 9q (*D9S910*), 11p (*D11S1984*), 13q (*D13S1493, D13S894,* and *D13S796*), and 17p (*D17S1303*; see Fig. 2 for examples of LOH). However, for only one marker (*D13S894* on 13q) was this difference statistically significant (odds ratio, 13.00; 95% confidence interval, 1.11–152.35; $P = 0.03$). Seven markers showed a lower frequency of LOH in group 1 than in group 2 (*D2S434, D3S4545, D3S1766, D6S1027, D9S1118, D14S587,* and *D15S655*), but none of these differences was statistically significant.

Clinical/Pathological Characteristics, Lifestyle Cancer Risk Factors, and Frequency of LOH. None of the four clinical/pathological characteristics examined was related to family history of esophageal cancer. Positive lymph nodes were associated with a significantly higher LOH frequency at *D6S1027* [LOH in 16 of 17 cases (94%) in those with positive lymph nodes, *versus* 9 of 19 cases (47%) in those with negative lymph nodes; odds ratio, 17.7; 95% confidence interval, 1.9–162.3. $P = 0.003$] and at *D9S910* [15 of 16 cases (94%) in those with positive lymph nodes, *versus* 9 of 16 cases (56%) in those with negative lymph nodes; odds ratio, 11.6; 95% confidence interval, 1.2–110.9; $P = 0.04$]. Higher LOH frequency at *D4S2361* was associated with lower pathological grade of esophageal cancer [6 of 6 (100%) for grade 1, 12 of 25 (48%) for grade 2, and 1 of 2 (50%) for grade 3; $P = 0.03$]. There were no significant associations between tumor location or stage and higher frequencies of LOH at any of the 18 markers.

There were no differences in the lifestyle cancer risk factors between patients with a family history of upper gastrointestinal cancer compared to those without a family history of upper gastrointestinal cancer. In addition, none of these risk factors was associated with a higher frequency of LOH at any of the 18 microsatellite markers examined.

DISCUSSION

Tumorigenesis is a multistep process that may involve oncogene activation and suppressor gene inactivation. The primary premise for the analysis of allelic loss in cancers is that areas of common genetic loss are likely to contain tumor suppressor genes. Allelic loss on chromosomes 3p, 5p, 5q, 9p, 9q, 11p, 13q, 17p, 17q, and 18q has been reported previously in esophageal cancer (12, 15–17). Our earlier genome-wide scan of esophageal squamous cell carcinomas from Shanxi province also found allelic loss in all 10 of these regions plus 7 additional regions (2q, 4p, 4q, 6q, 8p, 14q, and 15q) not reported previously (3).

To confirm the results of our genome-wide scan and expand our understanding of the molecular events and potential genetic susceptibility in esophageal cancer, we looked for allelic loss in the tumors of 46 additional patients, 23 with and 23 without a family history of upper gastrointestinal cancer. Overall, this expanded study confirmed our earlier observations of frequent allelic loss. For all 46 tumors combined, the lowest frequency of LOH for any of the 18 markers was 37% of informative tumors, whereas 15 markers showed LOH in ≥50% of the informative tumors, and 8 markers showed LOH in ≥75% of the informative tumors. Furthermore, the frequency of allelic loss in informative tumors for the 18 markers tested overall tended to be higher in persons with a positive family history.

On the basis of the significantly higher prevalence of LOH for *D13S894* found in this study in esophageal squamous cell carcinoma patients with a family history of upper gastrointestinal cancer in this study, chromosome 13q appears to be a promising region for further research. To date, two major tumor suppressor genes have been identified on chromosome 13, the retinoblastoma gene (*Rb1*) on 13q14.2, and the breast cancer susceptibility locus 2 (*BRCA2*) on 13q12.1 (18–20). Mutations of the *Rb1* gene appear to be rare in esophageal cancer (12, 21), and the frequency of *BRCA2* gene mutation in this tumor has not yet been investigated. We found frequent allelic loss for all three markers examined on chromosome 13q, especially in patients with a family history of upper gastrointestinal cancer. Because we assessed 18 different markers, the probability that one would be significant by chance alone is substantial and dictates a cautious interpretation and the need for further studies to con-

Table 1 Demographics, clinical/pathologic characteristics, and lifestyle cancer risk factors[a] of esophageal cancer patients

No.	ID no.	Age/ Sex	Tumor location	Patho-logical grade	Stage	Lymph node metastasis	Tobacco use	Alcohol consump-tion	Pickled vegetable consump-tion	Hot food consump-tion	Family history of upper gastrointestinal cancer[b]
A. Patients with a family history of upper gastrointestinal cancer (n = 23)											
1	SHE057	50/F	Middle	G2	3	Y	N	Weekly	Never	Never	EC (sister, 1994)
2	SHE066	51/M	Middle	G3	3	Y	Y	Never	Monthly	Daily	CC (father, 1971); EC (mother, 1977)
3	SHE081	58/M	Lower	G2	3	N	N	Weekly	Daily	Daily	EC (mother, 1968)
4	SHE138	55/F	Middle	G2	3	N	N	Never	Daily	Never	EC (mother, 1982)
5	SHE150	57/M	Middle	G1	3	N	Y	Weekly	Daily	Daily	EC (mother, 1957)
6	SHE152	44/F	Lower	G2	3	N	N	Never	Weekly	Daily	CC (mother, 1984)
7	SHE235	59/F	Upper middle	G2	3	N	Y	Weekly	Monthly	Monthly	EC (father, 1981)
8	SHE252	57/F	Middle	G2	3	Y	N	Never	Monthly	Monthly	5 EC (father, 1965; mother, 1957; sister, 1975; sister, 1986; paternal grandmother, 1922)
9	SHE263	50/M	Middle	G2	3	NA[c]	Y	Weekly	Weekly	Daily	3 EC (maternal uncle, 1988; maternal grandfather, 1948; maternal grandmother, 1971)
10	SHE265	49/F	Upper middle	G1	3	N	N	Never	Monthly	Daily	EC (father, 1974); BC (mother, 1974)
11	SHE328	50/F	Middle	G2	3	N	N	Never	Monthly	Monthly	2 EC (maternal uncle, 1975; maternal cousin, 1985)
12	SHE340	50/M	Lower	G2	3	Y	Y	Weekly	Monthly	Daily	CC (father, 1980); EC (paternal uncle, 1955)
13	SHE360	55/M	Middle	G3	3	Y	N	Never	Monthly	Daily	EC (father, 1954); BC (brother, 1973)
14	SHE384	53/F	Middle	G2	3	N	N	Never	Monthly	Daily	2 EC (father, 1946; sister); BC (brother)
15	SHE391	55/M	Middle	G2	3	N	N	Weekly	Weekly	Monthly	EC (father, 1971)
16	SHE408	65/M	Middle	G1	3	N	Y	Daily	Weekly	Daily	EC (paternal uncle's son, 1960)
17	SHE409	42/M	Middle	G2	3	N	Y	Weekly	Monthly	Monthly	EC (father, 1987)
18	SHE437	47/F	Middle	G2	3	N	N	Never	Monthly	Monthly	2 EC (father, 1970; mother, 1990)
19	SHE444	65/M	Lower	G2	3	N	Y	Never	Monthly	Monthly	EC (father, 1954)
20	SHE459	43/F	Lower	G2	3	N	N	Never	Monthly	Daily	EC (paternal aunt, 1984)
21	SHE480	65/M	Lower	G1	3	Y	Y	Daily	Monthly	Daily	EC (paternal uncle, 1996)
22	SHE495	54/M	Middle	G2	3	Y	Y	Weekly	Daily	Daily	EC (father, 1962)
23	SHE516	58/M	Middle	G2	3	N	N	Daily	Monthly	Monthly	2 EC (mother, 1955; brother, 1984)
B. Patients without a family history of upper gastrointestinal cancer (n = 23)											
1	SHE052	57/M	Middle	G2	3	N	N	Weekly	Daily	Never	N
2	SHE069	48/M	Middle	G3	3	N	Y	Never	Daily	Never	N
3	SHE095	58/M	Middle	G1	3	N	Y	Never	Daily	Daily	N
4	SHE096	57/M	Middle	G1	3	N	Y	Weekly	Monthly	Daily	N
5	SHE118	56/F	Middle	G3	3	Y	N	Never	Daily	Never	N
6	SHE170	52/F	Middle	G2	3	N	Y	Weekly	Daily	Monthly	N
7	SHE198	53/M	Middle	G2	3	Y	Y	Weekly	Daily	Weekly	N
8	SHE199	57/M	Middle	G2	3	N	Y	Daily	Daily	Never	N
9	SHE200	59/F	Middle	G2	3	Y	N	Weekly	Monthly	Daily	N
10	SHE208	56/F	Middle	G2	3	N	Y	Never	Daily	Daily	N
11	SHE216	43/F	Middle	G2	2	N	N	Weekly	Weekly	Daily	N
12	SHE240	51/M	Middle	G2	3	Y	Y	Daily	Weekly	Never	N
13	SHE247	45/M	Middle	G2	3	Y	Y	Never	Monthly	Daily	N
14	SHE261	57/M	Lower	G2	3	N	N	Weekly	Weekly	Monthly	N
15	SHE273	62/M	Middle	G2	3	Y	N	Weekly	Monthly	Monthly	N
16	SHE297	50/M	Middle	G2	3	Y	Y	Weekly	Daily	Monthly	N
17	SHE308	58/F	Middle	G3	3	Y	Y	Never	Never	Daily	N
18	SHE322	50/F	Middle + lower	G2	3	N	N	Never	Daily	Daily	N
19	SHE034	63/M	Lower	G2	3	Y	Y	Daily	Monthly	Daily	N
20	SHE488	48/F	Lower	G2	3	N	N	Never	Weekly	Daily	N
21	SHE497	60/M	Middle	G2	2	Y	Y	Weekly	Daily	Daily	N
22	SHE507	47/M	Middle	G2	3	Y	Y	Weekly	Monthly	Monthly	N
23	SHE510	59/M	Middle	G2	3	Y	Y	Daily	Monthly	Monthly	N

[a] Definitions in "Materials and Methods."
[b] EC, esophageal cancer; CC, cardia cancer; BC, body of stomach cancer.
[c] NA, not applicable.

Table 2 Summary of frequency of allelic loss in esophageal cancer patients with and without a family history of upper gastrointestinal cancer

Marker no.	Marker characteristics: Name	Chromosome location	Mean heterozygosity	Type	Size range	Frequency of allelic loss (tumors with allelic loss/total informative tumors) (%): Esophageal cancer patients with family history of upper gastrointestinal cancer (n = 23)	Esophageal cancer patients without family history of upper gastrointestinal cancer (n = 23)	P^a
1	*D2S434*	2q35	0.77	Tetra	262–286	5/14 (36)	6/16 (38)	1.000
2	*3S4545*	3p24.3–p25	0.82	Tetra	192–232	11/16 (69)	11/15 (73)	1.000
3	*D3S1766*	3p14–p21	0.76	Tetra	208–232	9/11 (82)	15/17 (88)	1.000
4	*D4S2632*	4p12–p14	0.81	Tetra	122–190	12/17 (71)	12/21 (57)	0.506
5	*D4S2361*	4q21.3–q22	0.74	Tri	149–164	10/16 (63)	9/17 (53)	0.728
6	*D5S2501*	5q21–q23.3	0.75	Tetra	314–334	8/12 (67)	6/14 (43)	0.267
7	*D6S1027*	6q21	0.77	Tri	117–138	10/16 (63)	16/21 (76)	0.475
8	*D8S1106*	8p21.2–p23.3	0.73	Tetra	127–151	13/16 (81)	8/14 (57)	0.236
9	*GATA62F03*	9p23–p24	0.64	Tetra	283–295	17/18 (94)	9/11 (82)	0.284
10	*9S1118*	9p11–q11	0.81	Tetra	141–177	16/18 (89)	17/18 (94)	1.000
11	*D9S910*	9q22.3–q31	0.66	Tri	105–129	13/17 (76)	11/15 (73)	1.000
12	*D11S1984*	11p15.3–p15.5	0.79	Tetra	166–206	13/16 (81)	12/16 (75)	1.000
13	*D13S1493*	13q11	0.80	Tetra	223–243	15/18 (83)	9/14 (64)	0.252
14	*D13S894*	13q12.3–q14.2	0.64	Tetra	180–200	13/14 (93)	4/8 (50)	0.039
15	*D13S796*	13q32–q34	0.80	Tetra	148–168	14/19 (74)	12/19 (63)	0.728
16	*D14S587*	14q12–q13	0.84	Tetra	250–278	6/18 (33)	7/17 (41)	0.733
17	*D15S655*	15q22–q23	0.72	Tri	234–252	3/11 (27)	6/13 (46)	0.423
18	*D17S1303*	17p11.2–p12	0.7	Tetra	225–245	13/13 (100)	17/19 (89)	0.502

[a] Calculated from two-sided Fisher's exact test.

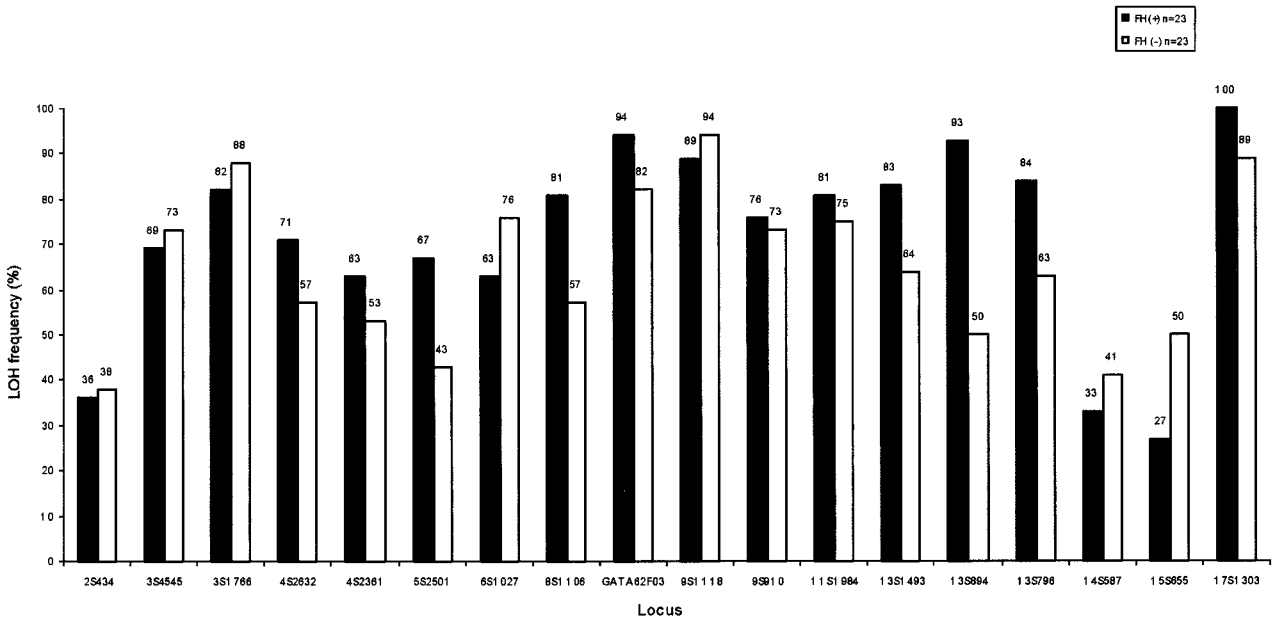

Fig. 1 Summary of LOH frequency in patients with (*FH+*) and without (*FH−*) a family history of upper gastrointestinal cancer.

firm the result. In addition, more markers flanking or adjacent to these markers should be tested in the future to expand these findings.

Allelic loss on chromosome 17p has been reported frequently in esophageal squamous cell carcinoma, as in many human tumors, and generally encompasses the *p53* locus at 17p13 (12, 22). We have not yet looked for mutation or LOH at *p53* but found frequent LOH for *D17S1303* (17p11.2-p12), a marker near *p53*. A high frequency of allelic loss on 17p11-p12 has also been reported in esophageal adenocarcinoma using two

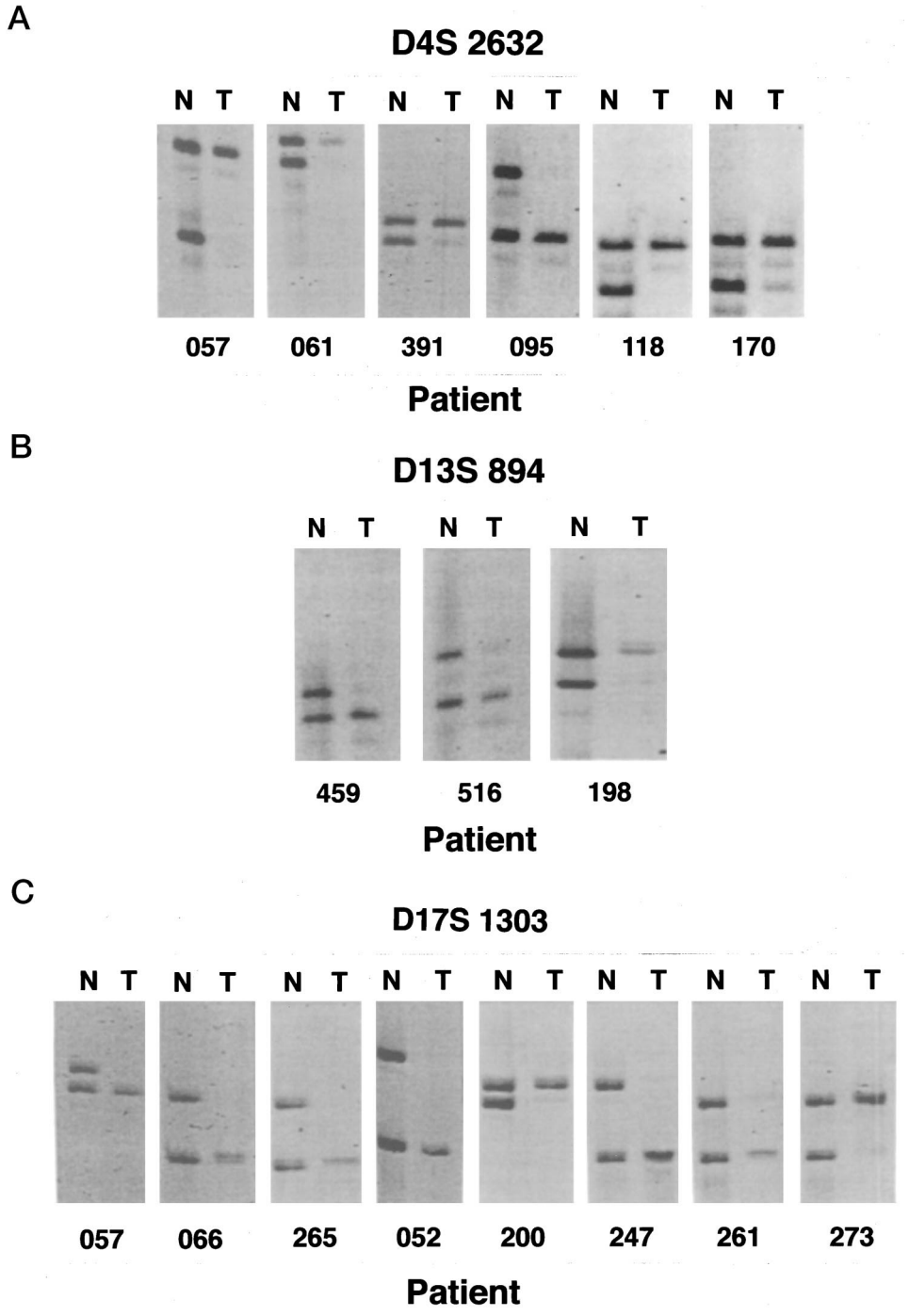

Fig. 2 Examples of LOH. *A, D4S2632; B, D13S894; C, D17S1303.*

markers located on 17p11-p12 (23). Further testing is required to determine whether our LOH findings on 17p are attributable to a mutation in *p53* or are attributable to the presence of an additional tumor suppressor gene in the 17p11-p12 region.

Frequent LOH was also seen in the present study, as in our genome-wide scan, for markers on chromosomes 11p (*D11S1984* at 11p15.3–15.5), 3p (*D3S1766* at 3p14-p21), 9p (*GATA62F03* at 9p23-p24), and 9q (*D9S1118* on 9p11-q11). A number of candidate genes are found on 11p (24–26), and allelic loss at the 11p15 locus has been observed in adenocarcinomas of the esophagus and stomach (23, 27). Frequent LOH on 3p, 9p, and 9q has been reported previously in esophageal cancer (12). These loci merit further exploration in esophageal and other cancers.

We also examined allelic loss in relation to available clinical/pathological characteristics and lifestyle cancer risk factors.

Among the clinical/pathological characteristics, allelic loss at *D6S1027* (6q21) and *D9S910* (9q22.3-q31) was more frequent in tumors that had metastasized than in those that had not, suggesting the possibility that genes in these regions could be modulating the metastatic process. In addition, LOH at locus *D4S2361* (4q21.3-q22) was inversely associated with tumor grade; patients with lower grade (G1) tumors showed more frequent LOH at *D4S2361* than those with higher grade (G2 and G3) tumors. We saw no association between allelic loss at any of the loci examined and any of the lifestyle cancer risk factors evaluated. Larger studies will be required to confirm our preliminary findings relating LOH at specific loci to metastasis and tumor grade and to explore more fully the relation between LOH and lifestyle cancer risk factors.

In conclusion, findings from this study refine our initial genome-wide scan of allelic loss in esophageal squamous cell carcinoma, confirming frequent allelic loss in 14 chromosomal regions and highlighting a locus on 13q where LOH is more common in patients with a family history of upper gastrointestinal cancer than in those without such history, suggesting that a gene in this area may be involved in genetic susceptibility to esophageal cancer. Markers potentially related to metastasis and tumor grade were also identified for future testing of genes located in these genomic regions.

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